Multiheme Cytochrome Mediated Redox Conduction through
Shewanella oneidensis MR-1 Cells

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ABSTRACT: Multiheme cytochromes function as extracellular electron transfer (EET) conduits that extend the metabolic reach of microorganisms to external solid surfaces. These conduits are also proposed to facilitate long-distance electron transport along cellular membranes and across multiple cells. Here we report electrochemical gating measurements of Shewanella oneidensis MR-1 cells linking interdigitated electrodes. The dependence of the source–drain current on gate potential demonstrates a redox conduction mechanism, which we link to the presence of multiheme cytochromes of the Mtr pathway. We also find that the measured thermal activation energy of 0.29 ± 0.03 eV is consistent with those obtained from electron hopping calculations through the S. oneidensis Mtr outer-membrane decaheme cytochromes. Our measurements and calculations have implications for understanding and controlling micrometer-scale electron transport in microbial systems.

Microorganisms have evolved extracellular electron transfer (EET) mechanisms to and from surfaces, allowing them to use redox-active minerals outside the cells as electron acceptors or donors for metabolism or to interact with other cells through interspecies electron transfer. In addition to their important environmental role in elemental cycling on a global scale, such microbes are being incorporated into electrodes as living catalysts for renewable energy technologies. Both native and engineered EET pathways allow microbes to drive fuel-to-electricity conversion on anodes and reduce CO₂ to fuels or other high value products on cathodes powered by renewable electricity.

In order to fully harness this biotechnological potential of microbe–electrode interactions, it is important to understand the underlying molecular pathways such as bacterial multiheme cytochromes that can allow EET across the biotic–abiotic interface. For example, the metal-reducing bacterium Shewanella oneidensis MR-1 relies on the multiheme MtrABC complex as the primary electron-conducting conduit across the cell envelope. At the cell surface, MtrC and a partnering decaheme cytochrome, OmcA, can act as terminal reductases for external electron-accepting surfaces or intermediary soluble redox shuttles.

In addition to allowing cellular respiration at electrode interfaces, EET components may also facilitate long-distance electron transport across neighboring cells. This proposal has been supported by conductivity measurements of Geobacter sulfurreducens biofilms, which can exceed tens of micrometers in thickness. The electrochemical signature of this conduction and its temperature dependence pointed to a mechanism of sequential electron transfer reactions through redox cofactors generally consistent with the hemes of cytochromes. However, it was not possible to implicate specific cytochromes, given that Geobacter possesses multiple parallel pathways across the cell envelope and through the biofilm, including more than 50 multiheme cytochromes and additional EET components such as conductive pili. These factors complicate the assignment of biofilm conduction to a specific molecular pathway.

Here we demonstrate cytochrome-specific micrometer-scale electron conductance through S. oneidensis cellular monolayers. S. oneidensis requires the Mtr pathway’s cytochromes for EET to electrodes and the now available crystal structures of the outer-membrane decaheme cytochromes (MtrF, MtrC, and OmcA) have recently enabled calculations of the thermodynamics and kinetics of electron transport along their heme chains. These molecular-level calculations now allow us to interpret measurements of cytochrome-mediated long-distance electron transport. Specifically, we report electrochemical measurements of conduction through S. oneidensis cell bridging electrodes. The conduction peaks at a gate potential corresponding to the formal potential of Mtr proteins, in support of a cytochrome-mediated multistep redox conduction mechanism similar to redox polymers. In addition, we find that this electron transport process is thermally activated, with an activation energy that is consistent with transport through MtrC and MtrF.

We cultivated S. oneidensis cells anaerobically in a defined medium as described previously before transfer to a bioelectrochemical reactor that contained an interdigitated microelectrode array (IDA) of indium tin oxide (ITO) source/cm².  

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drain electrode bands patterned on glass and separated by 5 μm gaps (Figure 1A). Both electrodes were first shorted and poised as a single anode (0.44 V vs SHE) for 16 h to promote cellular attachment.20 Fluorescent and atomic force microscopy revealed that cells accumulated on the IDA enough to span the source−drain gap in multiple locations (Figure 1B).

Following this electrochemical cultivation period, we probed EET and source−drain conduction using voltammetry and electrochemical gating.11 With both electrode bands still swept as a single electrode, turnover cyclic voltammetry (i.e., in the presence of lactate) revealed two catalytic waves as expected for S. oneidensis;20 the wave with an onset at −0.23 V corresponds to a flavin dependent mechanism, while that with 0.17 V onset is associated with EET via multiheme cytochromes (Figure S1). After medium exchange with fresh defined medium containing lactate but no electron acceptor, turnover voltammetry retained only the higher potential cytochrome-facilitated wave (Figure S1), as expected due to removal of the cell-secreted flavins.

Next, we performed electrochemical gating measurements to assess conduction through cells bridging the source and drain electrodes. Here, the source and drain potentials (E_S and E_D) are controlled by a bipotentiostat and scanned simultaneously at the same rate ν, while maintaining a constant small source−drain voltage (V_{SD} = E_D - E_S). Figure 2A features the measured currents at the source and drain electrodes, using V_{SD} = 0.02 V and scan rate, ν = 1 mV s⁻¹. Under both turnover and nonturnover conditions (no electron donor). Under nonturnover conditions, current at each electrode is determined by the V_{SD}-driven source−drain conduction current (I_{cond}), as well as a background contribution from Faradaic charging/discharging of cellular redox cofactors, but without turnover current from catalytic oxidation of lactate. The background contribution is eliminated by one of two methods: (i) subtracting raw source current from raw drain current to yield 2I_{cond}, which assumes the background current is the same for both electrodes in the limit of small V_{SD}, or (ii) performing separate V_{SD} = 0 scans to measure the Faradaic current and subsequently subtracting it from the current observed in the V_{SD} = 0.02 V scan at each electrode (see SI for details).11,12 We cross-checked both methods to show that they yield consistent results (Figure S2), indicating that the observed current peak reflects I_{cond} and not Faradaic current.

The gating procedure can be carried out at different temperatures (Figure 2B) to reveal the dependence of cellular
across the source sequential electron transfer steps are driven by a redox gradient (SHE) is consistent with a redox conduction mechanism where the expected formal potential of the cytochromes (\.\mbox{\.} E_{\text{a}} = 0.29 \pm 0.03 \text{ eV} (n = 3 separate biological replicates). \)

\[ I_{\text{cond}} = \frac{A}{T} e^{-E_a/(kT)} \]

This measured activation energy is consistent with values for single-step electron transfer in redox proteins, including cytochromes, especially when cofactors are partially solvent exposed, but is higher than that previously reported for transport in \textit{Geobacter} biofilms. Next, we compare our results to specific temperature-dependent stochastic calculations of electron hopping through the decaheme cytochromes MtrC and MtrF. These are the systems for which the thermodynamic parameters and electronic couplings that dictate individual electron transfer steps along their heme chains have been calculated. The \textquotedblleft staggered-cross\textquotedblright structure of the Mtr proteins potentially allows four terminal hemes (2, 5, 7, and 10) to serve as ingress/egress sites (Figure S6). While EET across the cellular membrane is thought to proceed between hemes 5 and 10 as terminals, the more buried hemes 2 and 7 may allow for interactions with soluble shuttles or additional cofactors such as flavins. Recent footprinting measurements also support a role for heme 7 as a donor to external minerals. To address multiple possibilities, we used a kinetic Monte Carlo (KMC) model to assess multiple routes by simulating bidirectional electron hopping with various ingress/egress sites (Figure S6). While EET across the cellular membrane is thought to proceed between hemes 5 and 10 as terminals, the more buried hemes 2 and 7 may allow for interactions with soluble shuttles or additional cofactors such as flavins. Recent footprinting measurements also support a role for heme 7 as a donor to external minerals.

To confirm the assignment of the conduction peak location to the formal potential of cytochromes, we performed voltammetry on an \textit{S. oneidensis} mutant (\textDelta Mtr/\textDelta mtrB/\textDelta mtrE) lacking eight functional periplasmic and outer-membrane cytochromes, including the entire Mtr–OmC pathway. As expected, the mutant did not exhibit the clear cytochrome oxidation or reduction peaks obvious in the wild-type (Figure S3). In addition, we tested any possible contribution of soluble components to conduction, by measuring the source–drain current of cell-free spent media obtained after cellular conduction measurements. Soluble factors, including these in the defined medium or cell-secreted redox active components (e.g., released cytochromes or Fe28), made a negligible contribution to the conduction current compared to cell-covered IDA (Figure S4). These experiments point to conduction primarily mediated by cell-associated multiheme cytochromes.

In Figure 3B, we show the dependence of $I_{\text{cond}}$ (at $E_{\text{G}} = E^0$) on temperature. By fitting to the expected dependence for an idealized redox conductor at small $V_{\text{SD}}$ (eq 1, where $A$ is a temperature-independent factor), we extract the thermal activation energy, $E_a = 0.29 \pm 0.03 \text{ eV} (n = 3$ separate biological replicates). \)

\[ I_{\text{cond}} = \frac{A}{T} e^{-E_a/(kT)} \]

The simulations were performed for 11 temperatures, between 286.15 and 306.15 K. Table 1 shows the overall thermal activation barriers for electron transport, with the majority of routes yielding activation barriers consistent with that measured through cells. Of the routes simulated in MtrC, the dominant terminal reductase for EET, the higher activation barriers of the 10$\leftrightarrow$5 route make it rate-limiting and are in very close agreement with the measured activation energy for cellular conduction ($0.29 \pm 0.03 \text{ eV}$). Recent observations of the distribution of \textit{Shewanella}\textquotesingle s periplasmic and outer-membrane cytochromes suggest a collision-exchange model, where long-distance transport is facilitated by a combination of electron hopping and intermediate diffusive steps (e.g., lateral cytochrome mobility) to link neighboring cytochromes. While not considering this full complexity nor accounting for OmC and periplasmic components, our calculations suggest that the temperature dependence of electron transport through electrode-spanning cells could

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**Figure 3.** Conduction measurements. (A) Dependence of conduction current, $I_{\text{cond}}$, on gate potential, $E_G$, for different temperatures (forward gating scans only shown for clarity; corresponding backward scans shown in Figure S5). (B) Representative Arrhenius-style plot (using peak $I_{\text{cond}}$ at $E_G = E^0$) yields an activation energy $E_a = 0.29 \pm 0.03 \text{ eV}$. 

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well be dictated by hopping along cytochrome heme chains rather than intermediate interprotein events.

To summarize, we have found that Shewanella oneidensis MR-1 cells can act as redox conduction channels across electrodes and identified multiheme cytochromes of the Mtr pathway as critical electron conduits that facilitate this conduction. These findings represent a step toward understanding and harnessing biological electron transport over cellular length scales.

ASSOCIATED CONTENT

# Supporting Information
The Supporting Information is available free of charge from the ACS Publications website at DOI: 10.1021/jacs.8b05104.

Experimental details, computational methodology, additional characterization, and controls (PDF)

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Notes

The authors declare no competing financial interest.

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Table 1. Activation Energies ($E_a$ in eV) Obtained from KMC Simulations of MtrC/F for Various Heme Ingress/Egress Routes

<table>
<thead>
<tr>
<th>flow route</th>
<th>$E_a$ MtrC</th>
<th>$E_a$ MtrF</th>
</tr>
</thead>
<tbody>
<tr>
<td>10→5 (one-electron)</td>
<td>0.299 ± 0.005</td>
<td>0.278 ± 0.005</td>
</tr>
<tr>
<td>5→10 (one-electron)</td>
<td>0.315 ± 0.007</td>
<td>0.231 ± 0.003</td>
</tr>
<tr>
<td>10→5 (two-electron)</td>
<td>0.309 ± 0.010</td>
<td>0.262 ± 0.003</td>
</tr>
<tr>
<td>5→10 (two-electron)</td>
<td>0.310 ± 0.018</td>
<td>0.278 ± 0.010</td>
</tr>
<tr>
<td>2→7</td>
<td>0.261 ± 0.016</td>
<td>0.268 ± 0.006</td>
</tr>
<tr>
<td>7→2</td>
<td>0.219 ± 0.007</td>
<td>0.272 ± 0.006</td>
</tr>
<tr>
<td>10→7</td>
<td>0.237 ± 0.007</td>
<td>0.202 ± 0.003</td>
</tr>
<tr>
<td>7→10</td>
<td>0.289 ± 0.010</td>
<td>0.279 ± 0.014</td>
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“One-electron” represents simulations using rates of transfer from the single electron hopping case. “Two-electron” corresponds to a similar scenario but with heme 7 always reduced. Equation 1 was used for fitting $E_a$.

REFERENCES